

ing the loose subcutaneous tissue in the form of a well-defined curved band, with the convexity generally directed forwards, it reaches and becomes continuous with the periosteum surrounding the distal extremity of the ungual phalanx. These two structures, viz., the ungual corium and the periosteum of the ungual phalanx, which are histologically very similar to one another, and distinct from the connective tissue forming the bulk of the terminal segment of the digit, are thus placed in complete continuity by means of the curved band of dense connective tissue above described.

V. "Experiments on the Action of Light on *Bacillus anthracis*."

By H. MARSHALL WARD, F.R.S., Professor of Botany, Royal Indian Engineering College. Received December 15, 1892.

It is abundantly evinced by experiments that direct insolation in some way leads to the destruction of spores of *Bacillus anthracis*, and in so far the results merely confirm what had already been discovered by Downes and Blunt in 1877 and 1878.*

From the fact that an apparent retardation of the development of the colonies on plates exposed to light was observed several times under circumstances which suggested a direct inhibitory action of even ordinary day-light, the author went further into this particular question with results as startling as they are important, for if the explanation given of the phenomena observed in the following experiments turns out to be the correct one, we stand face to face with the fact that by far the most potent factor in the purification of the air and rivers of bacteria is the sun-light. The fact that direct sun-light is efficacious as a bactericide has been long suspected, but put forward very vaguely in most cases.

Starting from the observation that a test-tube, or small flask, containing a few c.c. of Thames water with many hundreds of thousands of anthrax spores in it may be entirely rid of living spores by continued exposure daily for a few days to the light of the sun, and that even a few weeks of bright summer day-light—not direct insolation—reduces the number of spores capable of development on gelatine, it seemed worth while to try the effect of direct insolation on plate-cultures, to see if the results could be got more quickly and definitely.†

Preliminary trials with gelatine plate-cultures at the end of the

* See p. 237 of "First Report to the Water Research Committee of the Royal Society" ('Roy. Soc. Proc.,' vol. 51, 1892) for the literature on this subject up to 1891.

† It appears that Buchner ('Centr. f. Bakt.,' vol. 12, 1892) has already done this for typhoid, and finds the direct rays of the summer sun quite effective.

summer soon showed that precautions of several kinds were necessary. The direct exposure of an ordinary plate-culture to the full light of even a September or October sun, especially in the afternoon, usually leads at once to the running and liquefaction of the gelatine, and although the exposed plates eventually showed fewer anthrax colonies than similar plates not exposed, the matter was too complicated to give satisfactory results. Obviously one objection was that the spores might have begun to germinate, and the young colonies killed by the high temperatures.

Experiments made in October with gelatine plates wrapped in black paper, in which a figure—a square, cross, or letter—was cut, also led to results too indefinite for satisfaction, although it was clear in some cases that if the plates lay quite flat, the illuminated area was on the whole clear of colonies, while that part of the plate covered by the paper was full of colonies.

But another source of vexation arose. After the plates had been exposed to the sunlight for, say, six hours, it was necessary to put them in the incubator ($20-22^{\circ}$ C. was the temperature used) for two days or so, to develop the colonies, and in many cases it was observed that by the time the colonies were sufficiently far advanced to show up clearly, liquefaction had extended so far as to render the figure blurred and doubtful.

Stencil plates of zinc were employed with, at first, equally uncertain results. The stencil plate was fixed to the bottom of the plate culture, outside, and every other part covered with blackened paper: the plate was then placed on a level surface, the stencil-covered face upward, and exposed to the direct sunlight. As before, the gelatine softened and in many cases ran, and the results were uncertain, though not altogether discouraging.

In November it was found that more definite results could be obtained, and the problem was at last solved.

Meanwhile it had already been found possible to obtain sun prints in the following way with agar plates. Ordinary agar was heated and allowed to cool to between 50° and 60° C., and was then richly infected with anthrax spores, and made into plates as usual. Such plates were then covered with a stencil plate on the lower face—the stencil plate being therefore separated from the infected agar only by the glass of the plate—and wrapped elsewhere closely in dull black paper, so that, on exposure to the sun, only the cut-out figure or letter allowed the solar rays to reach the agar.

Such plates were then exposed to the direct rays of the October sun for from two to six hours; or they were placed on the ring of a retort-stand, stencil downwards, and the sun-light reflected upwards from a plane mirror below.

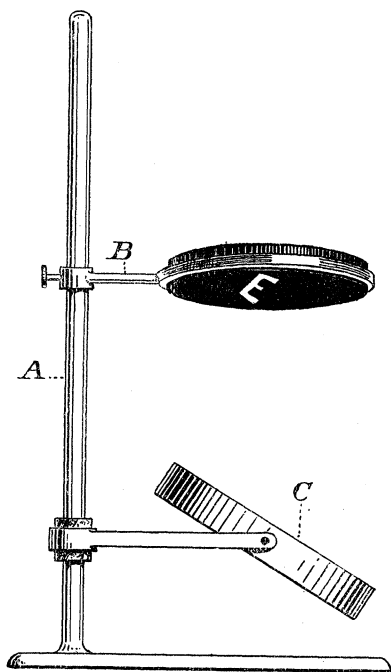
After the insolation, these plates were incubated for at least forty-

eight hours at 20° C., and on removing the wrappers the colonies of anthrax were found densely covering all parts of the plate except the area—a letter or cross, &c.—exposed to the sun-light. There, however, the spores were killed, and the agar remained perfectly clear, showing the form of a sharp transparent letter, cross, &c., in a ground-work rendered cloudy and opaque by the innumerable colonies of anthrax.

Experiments proved that this was not due to high temperature, for a thermometer with its bulb next the insolated glass rarely rose beyond 14° to 16° C., and never beyond 18° C., and even if the thermometer did not record the temperature inside the plate, this can scarcely have been much higher.

As long as this latter point remained uncertain, however, the experiments could not be regarded as satisfactory; whence it was necessary to again have recourse to gelatine cultures. The gelatine employed began to run at 29° C., and in November it was found that such plates exposed outside, either to directly incident sunshine, or to directly reflected rays, showed a temperature of 12° to 13° C. at the insolated glass surface, and even five to six hours' exposure caused no running of the gelatine.

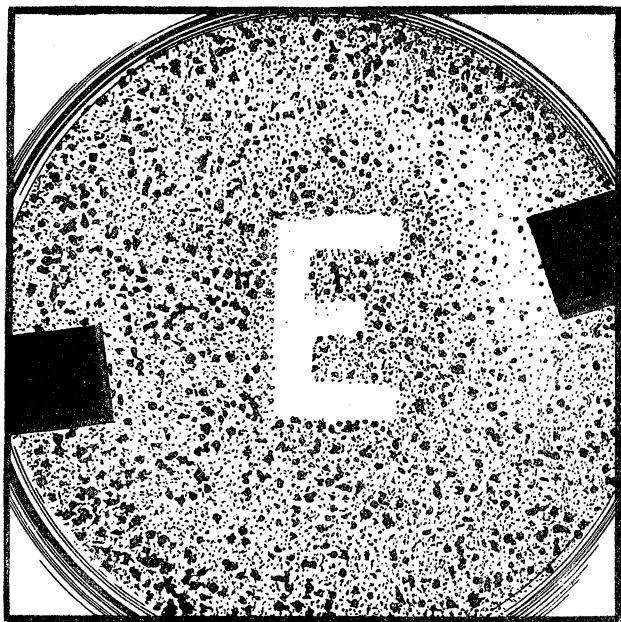
FIG. 1.



The following experiment may be selected as a type of the rest:—A (fig. 1) is the upright of an ordinary retort-stand; on the ring B rested a gelatine plate-culture of anthrax spores, covered with black paper everywhere except the cut-out letter E, seen on its lower face. C was an ordinary plane microscope-mirror, with its arm fitted to a cork on A.

The whole was placed in the middle of a field at Cooper's Hill at 9.30 A.M. on Wednesday, November 30, and exposed to the clear, but low, sunshine which prevailed that day, the mirror being so arranged (from time to time as necessary) as to reflect the light on the E the whole period, until 3.30 P.M., when the plate was removed and placed in the dark incubator at 20° C. On the following Friday—*i.e.*, after less than forty-eight hours' incubation—the letter E stood out sharp and clearly transparent from the faint grey of the rest of the plate of gelatine. Not a trace of anthrax could be found in the clear area, even with the microscope, while the grey and almost opaque appearance of the rest of the plate was due to innumerable colonies of that organism which had developed in the interval.

FIG. 2.



It was impossible to incubate the plate longer for fear of liquefaction, whence the sceptical may reply that the anthrax exposed to light was only retarded; the experiments with agar show that such

is not the case, however, and that if the insolation is complete the spores are rendered incapable of germinating at all, as proved by removing pieces of the clear agar or gelatine and attempting to make tube cultures from them: in all cases where insolation is complete they remain sterile.

The chief value of these gelatine plate exposures in November, however, is that they prove conclusively (1) that the rays of a *winter* sun are capable, even if reflected, of killing the spores, and (2) that it is really the solar rays which do this directly, and not any effect of a higher temperature, since the gelatine remains solid throughout.

Experience has shown, however, that some precautions are necessary in selecting the anthrax cultures employed for these experiments with gelatine. The light certainly retards or kills (according to its intensity or the length of exposure) virulent spores, but if one takes the spores, mixed with vegetative bacilli, direct from a thoroughly liquefied gelatine culture, or from a bouillon culture, the plates are apt to be liquefied too rapidly for the proper development of the light print, evidently because so much of the liquefying enzyme is carried in when inoculating the plates. The same danger is run when active bacilli alone are employed.

The best method of avoiding these disadvantages has been found to be the following, and it has the additional merit of enabling us to prove, beyond all doubt, that the ripe spores of *Bacillus anthracis* are really inhibited or killed by sunlight.

A few c.c. of sterile distilled water in a tube are thoroughly saturated with the anthrax spores taken from an old culture which has never been exposed to light, and the tube placed for twenty-four hours at 56° C.; this kills all immature spores, bacilli, and enzymes, and leaves us with a crop of the most resistant and fully matured virulent spores.

Experiments with such spores have been made to determine the relative power of the different rays of the spectrum to destroy the anthrax.

It is necessary to note first, however, that in experimenting with the electric light, although but few exposures have been made as yet, it is evident that its effects are feebler than those of the winter sun.

At present it has only been possible to observe that the inhibiting effects are stronger at the blue end of the spectrum than at the red, and exposures to sunlight passing through coloured glasses confirm this result; but the observations are being continued in the hope of getting a perfectly sharp record of the effects of each set of rays.

The following series of experiments are quoted in detail, because they teach several details of importance, in addition to proving the main fact.

On December 7 three gelatine plates and five agar plates were

prepared with spores from a very vigorous and virulent agar tube of anthrax. The spores, which were quite mature, were not subjected to heat, but simply shaken in sterile water to wash and separate them thoroughly.

The three gelatine plates were made at 35° C., the agar plates at 60° C., neither of which temperatures could injure the ripe spores.

The three gelatine plates were labelled *p* 1, *p* 2, and *p* 3, and the agar plates *p* 4 to *p* 8 in order.

Immediately after making the plates, all were exposed to the December sun, except plates *p* 4, *p* 5, and *p* 6, and this was done as follows:—In each case the plate had a stencil plate with a cut-out letter on its lower face, and arranged as described above (p. 396).

p 1, a gelatine plate with a *large* letter M, was exposed, face down, to the light reflected from a mirror (see fig. 1) for three hours on December 7, and for four hours on December 8, the interval being passed in a cold room (*t* about 8—9° C.), and then incubated at 20° in the dark.

p 8 was treated in exactly the same manner. But this was an agar plate with a *large* W.

p 2, a gelatine plate with a *large* H, was exposed and heated in the same way, except that no mirror was used, the latter being upwards towards the sun.

p 3, a gelatine plate with a *large* B, was similarly exposed, face up, but a plane mirror arranged to reflect light down upon it.

p 7, an agar plate with a *large* E, was treated exactly as the last.

There now remain the three agar plates, *p* 4, *p* 5, and *p* 6, to account for.

p 4 was placed forthwith in the dark incubator at 20° C.

p 5 and *p* 6 were kept for eighteen hours in a drawer, the average temperature of which is almost 16° C., and were not exposed till next day (December 8), when they lay for five hours, face upwards, and with a mirror above them. *p* 5 had a *small* E, and *p* 6 a broad but small I, to let the light in.

After exposure, these also were put in the same incubator with the others.

Nothing was visible to the unaided eye on these plates (except *p* 4) until the 11th instant, though the microscope showed that germination was proceeding on the 10th. The plate *p* 4, however, had a distinct veil of colonies all over it on the 9th, and this had developed to a dense typical growth by the 11th.

On December 11, at 10 A.M., the state of affairs, as regards the exposed plates, was as follows:—

p 5 and *p* 6 showed each a sharp transparent letter—E and I respectively—of clear agar in a dull grey matrix of strong anthrax colonies, which covered all the unexposed parts of the plate.

p 1, *p* 2, and *p* 3 showed in each case a perfectly clear central patch, about $1\frac{1}{2}$ inches diameter, with anthrax colonies in the gelatine around. These anthrax colonies were the *larger and more vigorous the more distant they were from the clear centre*. In other words, the anthrax spores had begun to germinate, and the colonies were growing more vigorously, in centripetal order.

On *p* 7 and *p* 8 germination was beginning, but the colonies were as yet too young to enable one to judge of the results.

The first point of interest is to account for the pronounced results in the plates *p* 5 and *p* 6, and the want of sharp outlines in *p* 1, *p* 2, and *p* 3, and the explanation seems to be that, owing to the plates 5 and 6 having laid over night at 16° C., the spores began slowly to germinate out, *and were consequently in their most tender condition when exposed to the sunlight next day*.

The peculiar centripetal order of development of the colonies on plates *p* 1, *p* 2, and *p* 3 gave rise to the following attempt at explanation. After observing that the clear space in the middle was not due to the centre of the plate being raised, and the infected gelatine having run down to the periphery—a possible event with some batches of Petrie's dishes—it was surmised that the *large* letters employed might give the clue.

This was found to be the case. The solar rays on entering the plate were largely reflected from the glass lid of the plates, and so produced feebler insolation effects on parts of the plate around the letter: these effects were naturally feebler and feebler towards the margin, and so the inhibitory action became less pronounced at distances further and further removed from the centre. Those spores, therefore, which were nearest the periphery germinated out first, and those nearer the centre were retarded more and more in proportion to their proximity to the insulated letter.

That this is the correct interpretation of the facts follows clearly from the further behaviour of the above plates.

At 10 P.M. on the 11th—*i.e.*, twelve hours after the morning examination—the plates *p* 1, *p* 2, and *p* 3 exhibited their respective letters M, H, and B quite clearly, in the grey matrix of anthrax which had rapidly developed in the interval, and excepting a slight want of sharpness in the H of *p* 2, the results could hardly have been more satisfactory.

In *p* 7 and *p* 8 the *very faint* outlines of the letters were also showing.

On the 12th, at 8.30 A.M., the gelatine plates had begun to run, and although the M of *p* 1 was still intact, and very well marked, *p* 2 had liquefied completely, so that the H was a clear patch with blurred outlines in the centre. *p* 3 still showed the outlines of the B, but it was impossible to keep it longer.

The main point was definitely established, however, and the treatment of the plates proves conclusively that the spores are not killed by high or low temperatures, *but by the direct solar rays.*

These experiments are being continued in order to answer some other questions in this connexion.

The gelatine and agar after such exposures as have been described are still capable of supporting a growth of *B. anthracis* if fresh spores are sown on them, whence the effects described are not merely due to the sub-strata being spoilt as food material.

The Society adjourned over the Christmas Recess to Thursday, January 19, 1893.

Presents, December 15, 1892.

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FIG. 1.

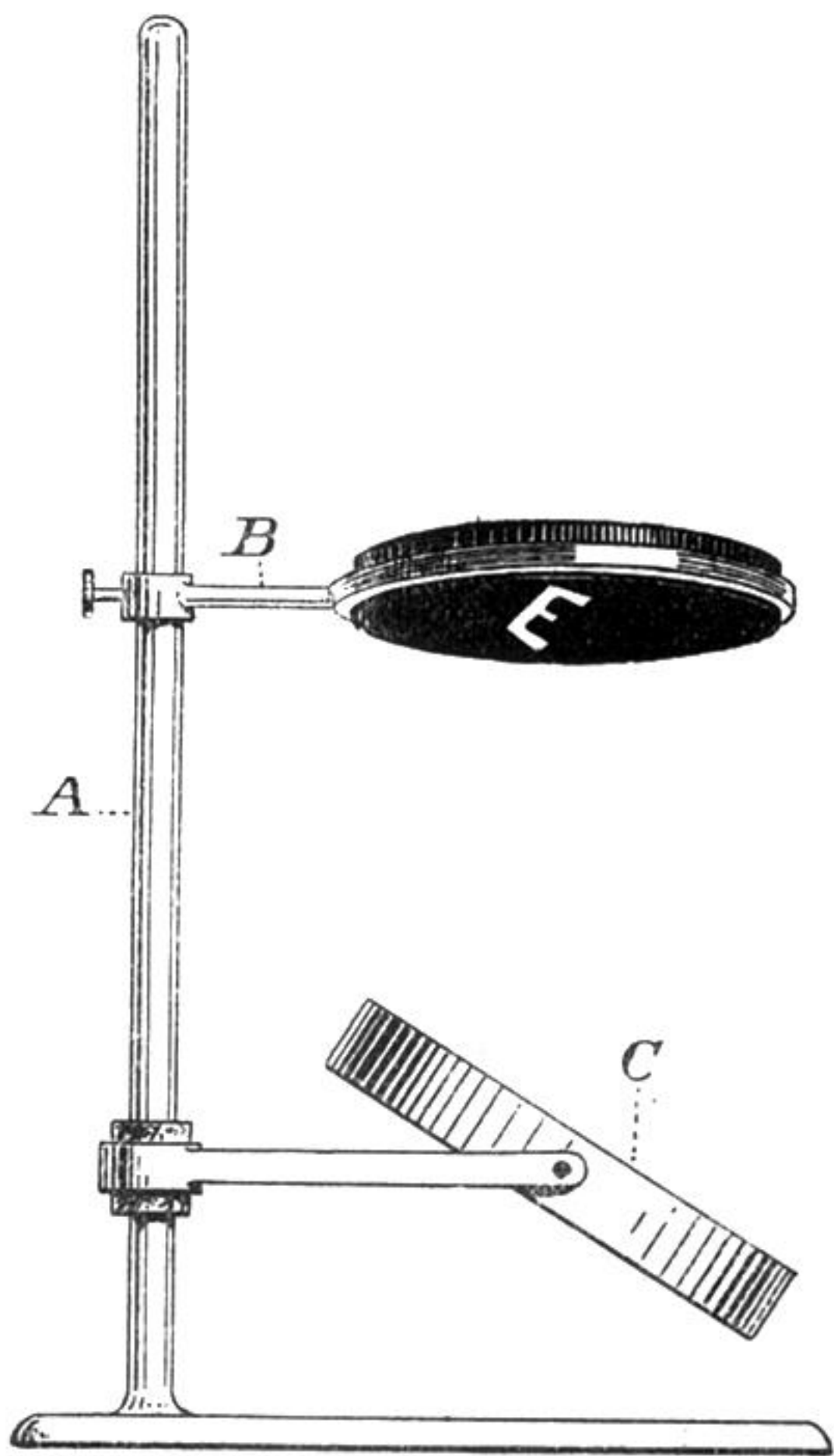


FIG. 2.

